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     (FILE 'HOME' ENTERED AT 14:50:39 ON 12 NOV 2001)

     FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT
     14:57:00 ON 12 NOV 2001
L1            78 S (METHIONINE (W) SYNTHASE (W) REDUCTASE) OR ((METHININE (W) SY
L2            65 S L1 AND (MAMMALIAN OR HUMAN OR SAPIENS OR MURINE)
L3            33 DUP REM L1 (45 DUPLICATES REMOVED)
L4             2 S L3 NOT PY>1998

     FILE 'CAPLUS' ENTERED AT 15:03:26 ON 12 NOV 2001
                E GRAVEL R A/AU 25
L5            14 S (E3 OR E8 OR E9) AND (METHIONINE)
L6             9 S L5 NOT PY>1998

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L6 ANSWER 1 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:571874 CAPLUS
DOCUMENT NUMBER: 129:314534
TITLE: Functionally null mutations in patients with the cblG-variant form of **methionine** synthase deficiency
AUTHOR(S): Wilson, A.; Leclerc, D.; Saberi, F.; Campeau, E.; Hwang, H. Y.; Shane, B.; Phillips, J. A., III; Rosenblatt, D. S.; **Gravel, R. A.**
CORPORATE SOURCE: Medical Research Council Group in Medical Genetics, Montreal Children's Hospital, McGill University, Montreal, Can.
SOURCE: Am. J. Hum. Genet. (1998), 63(2), 409-414
CODEN: AJHGAG; ISSN: 0002-9297
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Methionine** synthase (MS) catalyzes the methylation of homocysteine to **methionine** and requires the vitamin B12 deriv., methylcobalamin, as cofactor. The authors and others have recently cloned cDNAs for MS and described mutations assocd. with the cblG complementation group that correspond to MS deficiency. A subset of cblG, known as "cblG variant," shows no detectable MS activity and failure of [57Co]CN cobalamin to incorporate into MS in patient fibroblasts. The authors report the mutations responsible for three cblG-variant patients, two of them siblings, who presented with neonatal seizures, severe developmental delay, and elevated plasma homocysteine. Cell lines from all three patients were neg. by northern blotting, though trace MS mRNA could be detected by phosphorimage anal. Reverse transcriptase-PCR, SSCP, and nucleotide sequence anal. revealed four mutations. All were functionally null, creating either a frameshift with a downstream stop codon or an insert contg. an internal stop codon. Of the two mutations found in the siblings, one of them, intervening sequence (IVS)-166A.fwdarw.G, generates a cryptic donor splice site at position -166 of an intron beginning after Leu113, resulting in a 165-bp insertion of intronic sequence at junction 339/340. The second is a 2-bp deletion, 2112delTC. Mutations in the third patient include a G.fwdarw.A substitution, well within the intron after Lys203, which results in intronic inserts of 128 or 78 bp in the mRNA. The second mutation is a 1-bp insertion, 3378insA. The authors conclude that the absence of MS protein in these cblG variants is due to mutations causing premature translation termination and consequent mRNA instability.

L6 ANSWER 2 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:209132 CAPLUS
DOCUMENT NUMBER: 128:318758
TITLE: Cloning and mapping of a cDNA for **methionine** synthase reductase, a flavoprotein defective in patients with homocystinuria
AUTHOR(S): Leclerc, D.; Wilson, A.; Dumas, R.; Gafuik, C.; Song, D.; Watkins, D.; Heng, H. H. Q.; Rommens, J. M.; Scherer, S. W.; Rosenblatt, D. S.; **Gravel, R. A.**
CORPORATE SOURCE: Medical Res. Council Group Medical Genetics, Montreal Children's Hosp., McGill Univ. Health Cent., Montreal, PQ, H3Z 2Z3, Can.
SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1998), 95(6), 3059-3064
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Methionine** synthase catalyzes the remethylation of homocysteine to **methionine** via a reaction in which methylcobalamin serves as an intermediate Me carrier. Over time, the cob(I)alamin cofactor of **methionine** synthase becomes oxidized to cob(II)alamin rendering the enzyme inactive. Regeneration of functional enzyme requires reductive methylation via a reaction in which S-adenosylmethionine is utilized as a Me donor. Patients of the cblE complementation group of disorders of folate/cobalamin metab. who are defective in reductive activation of **methionine** synthase exhibit megaloblastic anemia, developmental delay, hyperhomocysteinemia, and hypomethioninemia. Using consensus sequences to predicted binding sites for FMN, FAD, and NADPH, a cDNA corresponding to the "**methionine** synthase reductase" reducing system required for maintenance of the **methionine** synthase in a functional state was cloned. The gene MTRR has been localized to chromosome 5p15.2-15.3. A predominant mRNA of 3.6 kb is detected by Northern blot anal. The deduced protein is a novel member of the FNR family of electron transferases, contg. 698 amino acids with a predicted mol. mass of 77,700. It shares 38% identify with human cytochrome P 450 reductase and 43% with the C. elegans putative **methionine**

synthase reductase. The authenticity of the cDNA sequence was confirmed by identification of mutations in cblE patients, including a 4-bp frameshift in two affected siblings and a 3-bp deletion in a third patient. The cloning of the cDNA will permit the diagnostic characterization of cblE patients and investigation of the potential role of polymorphisms of this enzyme as risk factor in hyperhomocysteinemia-linked vascular disease.

L6 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1997:673231 CAPLUS
DOCUMENT NUMBER: 127:327278
TITLE: The **methionine** synthase (Mtr) gene maps to proximal mouse chromosome 13
AUTHOR(S): Zhang, Zhi-Xin; Leclerc, Daniel; **Gravel, Roy** ; Rozen, Rima
CORPORATE SOURCE: Departments Human Genetics, Pediatrics Biology, McGill University-Montreal Children's Hospital Research Institute, Montreal, PQ, H3Z 2Z3, Can.
SOURCE: Mamm. Genome (1997), 8(10), 787-788
CODEN: MAMGEC; ISSN: 0938-8990
PUBLISHER: Springer
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The Mtr gene was localized by RFLP anal. of 96 animals from an interspecific backcross panel provided by the Jackson Lab., Bar Harbor, Me. The enzyme (EC 2.1.1.13) catalyzes homocysteine remethylation to **methionine**, with 5-methyltetrahydrofolate as the Me donor and methylcobalamin as a cofactor. The gene was mapped to 1q34. Studies involving the mouse **methionine** synthase gene will be useful in assessing the role of this important enzyme in the development of birth defects and/or vascular disease.

L6 ANSWER 4 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1996:756651 CAPLUS
DOCUMENT NUMBER: 126:29967
TITLE: Human **methionine** synthase: cDNA cloning and identification of mutations in patients of the cblG complementation group of folate/cobalamin disorders
AUTHOR(S): Leclerc, D.; Campeau, E.; Goyette, P.; Adjalla, C. E.; Christensen, B.; Ross, M.; Eydoux, P.; Rosenblatt, D. S.; Rozen, R.; **Gravel, R. A.**
CORPORATE SOURCE: MRC Group Med. Genet., McGill Univ., Montreal, PQ, H3Z 2Z3, Can.
SOURCE: Hum. Mol. Genet. (1996), 5(12), 1867-1874
CODEN: HMGEE5; ISSN: 0964-6906
PUBLISHER: Oxford University Press
DOCUMENT TYPE: Journal
LANGUAGE: English
AB **Methionine** synthase catalyzes the remethylation of homocysteine to **methionine** in a methylcobalamin-dependent reaction. The authors used specific regions of homol. within the **methionine** synthase sequences of several lower organisms to clone a human **methionine** synthase cDNA by a combination of RT-PCR and inverse PCR. The enzyme is 1265 amino acids in length and contains the seven residue structure-based sequence fingerprint identified for cobalamin-contg. enzymes. The gene was localized to chromosome 1q43 by the FISH technique. The authors have identified one missense mutation and a 3 bp deletion in patients of the cblG complementation group of inherited homocysteine/folate disorders by SSCP and sequence anal., as well as an amino acid substitution present in high frequency in the general population. The authors discuss the possibility that a mild deficiency of **methionine** synthase activity could be assocd. with mild hyperhomocysteinemia, a risk factor for cardiovascular disease and possibly neural tube defects.

L6 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1989:529709 CAPLUS
DOCUMENT NUMBER: 111:129709
TITLE: Human mitochondrial propionyl-CoA carboxylase: localization of the N-terminus of the pro- and mature .alpha. chains in the deduced primary sequence of a full-length cDNA
AUTHOR(S): Lamhonwah, Anne Marie; Mahuran, Don; **Gravel, Roy A.**
CORPORATE SOURCE: Res. Inst., Hosp. Sick Child., Toronto, ON, M5G 1X8, Can.
SOURCE: Nucleic Acids Res. (1989), 17(11), 4396
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A full-length, 2478-nucleotide cDNA (pPCCA32) coding for the .alpha., biotin-contg. polypeptide, of propionyl-CoA carboxylase (EC 6.4.1.3) is

reported. It contains a 5'-untranslated sequence of 48 nucleotides, an open reading frame of 2106 nucleotides coding for a 702 amino acid pre-.alpha. polypeptide with the biotin-binding site at residue 668 and a 3'-untranslated sequence of 246 nucleotides with the consensus polyadenylation AATAAA (underlined) upstream of a poly(A) tail. A match of 28 amino acids with the N-terminal amino acid sequence detd. from the mature .alpha. polypeptide of purified human liver enzyme, sepd. from the .beta. polypeptide by HPLC, was found in the deduced sequence of the cDNA. Two **methionine** residues (in bold) are located in the putative mitochondrial leader sequence of 26 amino acids. As typical of such sequences, this region is hydrophobic and arginine-rich.

L6 ANSWER 6 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:403006 CAPLUS
DOCUMENT NUMBER: 109:3006
TITLE: Localization of the pro-sequence within the total deduced primary structure of human .beta.-hexosaminidase B
AUTHOR(S): Stirling, John; Leung, Amy; **Gravel, Roy A.**; Mahuran, Don
CORPORATE SOURCE: Dep. Biochem., Kings Coll., London, UK
SOURCE: FEBS Lett. (1988), 231(1), 47-50
CODEN: FEBLAL; ISSN: 0014-5793
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The .beta. subunit of .beta.-acetylhexosaminidase (EC 3.2.1.52) is synthesized in the rough endoplasmic reticulum as a prepropolypeptide. After the loss of the signal peptide and formation of an enzymically active dimer, the proenzyme is either secreted from the cell or transported into the lysosome for processing to its mature form. In order to characterize the early posttranslational events 1 mg of prohexosaminidase B was purified from the NH4Cl contg. medium of fibroblasts derived from a patient with the infantile form of Tay-Sachs disease. The partial N-terminal sequence was mapped to a position 42 residues C-terminal to the 1st in-frame ATG (**methionine** residue) and 79 residues N-terminal to the known mature N-terminus. This position corresponds to that predicted for the cleavage of a 17 amino acid signal peptide generated through the use of the 3rd rather than the 1st in-frame ATG as the initiation site for protein synthesis.

L6 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1988:200871 CAPLUS
DOCUMENT NUMBER: 108:200871
TITLE: Proteolytic processing of pro-.alpha. and pro-.beta. precursors from human .beta.-hexosaminidase. Generation of the mature .alpha. and .beta.a.beta.b subunits
AUTHOR(S): Mahuran, Don J.; Neote, Kuldeep; Klavins, Maris H.; Leung, Amy; **Gravel, Roy A.**
CORPORATE SOURCE: Res. Inst., Hosp. Sick Child., Toronto, ON, M5G 1X8, Can.
SOURCE: J. Biol. Chem. (1988), 263(10), 4612-18
CODEN: JBCHA3; ISSN: 0021-9258
DOCUMENT TYPE: Journal
LANGUAGE: English
AB There are 2 major isoenzymes of human lysosomal .beta.-hexosaminidase (EC 3.2.1.52), hexosaminidase A, .alpha.(.beta.a,.beta.b), and hexosaminidase B, 2(.beta.a.beta.b). The .alpha. subunit contains a single polypeptide chain, while the .beta. subunit is composed of 2 nonidentical chains (.beta.a and .beta.b) derived from a common pro-.beta. precursor. In order to define the structure of the .alpha. and .beta. subunits generated in the lysosome, the .alpha., .beta.a, and .beta.b polypeptides of hexosaminidase A and B were sepd. by a combination of mol. sieve and ion exchange HPLC, and N-terminal sequences were detd. These were localized to the deduced amino acid sequences of previously isolated cDNAs coding for the prepro-.alpha. and .beta. polypeptides. From this anal., the sites of hydrolysis generating the mature .alpha., .beta.a, and .beta.b chains were detd. First, the signal peptide required for processing of the prepropolypeptides through the rough endoplasmic reticulum was predicted from the first in-frame **methionine** residue on the cDNA. Second, amino acid sequencing defined the N-termini of the mature polypeptide chains and identified the pro-sequences removed from both the pro-.alpha. and pro-.beta. polypeptides. Third, an internal cleavage resulted in the removal of a tetrapeptide, Arg-Gln-Asn-Lys, and tripeptide, Arg-Gln-Asn, from the pro-.beta. chain of hexosaminidase A and B, resp., to generate the .beta.b and .beta.a chains. This result localized the .beta.b and .beta.a chains to the N-terminal and C-terminal halves of the pro-.beta. sequence, resp. Minimal or no C-terminal processing of the pro-.beta. chain in the lysosome was previously obsd. However, there is trimming at the C terminus of the pro-.alpha. chain based on comparison of mol. wts. of deglycosylated .alpha. with the isolated .beta.b and .beta.a chains comprising the mature .beta. subunit

with those predicted from the cDNA. Thus, in the lysosome the pro forms of hexosaminidase A and B undergo extensive proteolytic processing which, while specific in nature, has the appearance of removing easily accessible, nonessential domains, rather than contributing to biosynthetic maturation of function.

L6 ANSWER 8 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:588871 CAPLUS
DOCUMENT NUMBER: 99:188871
TITLE: Assignment of the .alpha. and .beta. chains of human propionyl-CoA carboxylase to genetic complementation groups
AUTHOR(S): Lam Hon Wah, A. M.; Lam, K. F.; Tsui, F.; Robinson, B.; Saunders, M. E.; **Gravel, R. A.**
CORPORATE SOURCE: Res. Inst., Hosp. Sick Child., Toronto, ON, M5G 1X8, Can.
SOURCE: Am. J. Hum. Genet. (1983), 35(5), 889-99
CODEN: AJHGAG; ISSN: 0002-9297
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Propionic acidemia is a metabolic disorder resulting from a deficiency of propionyl-CoA carboxylase [9023-94-3] activity. The enzyme is composed of 2 polypeptides: a 72,000-dalton .alpha. chain which contains the biotin ligand and a 56,000-dalton .beta. chain. It has been suggested that the 2 major complementation groups in this disorder, pccA and pccBC (with subgroups pccB and pccC), correspond to the genes encoding these 2 chains. To correlate gene products with complementation groups, 15 mutant and 4 normal human fibroblast strains were analyzed by [35S]**methionine** and [3H]biotin labeling. Immunopptn. and gel electrophoresis of the polypeptides revealed that .alpha. chains are synthesized by mutants of pccBC and both subgroups but not in 4 of 5 pccA mutants. .beta. Chains were detected only in pccB mutants. Apparently, pccA encodes the .alpha. chain of PCC while pccBC encodes the .beta. chain; .beta. chain instability in the absence of the .alpha. chain is predicted.

L6 ANSWER 9 OF 9 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1983:158327 CAPLUS
DOCUMENT NUMBER: 98:158327
TITLE: Characterization of polypeptides serologically and structurally related to hexosaminidase in cultured fibroblasts
AUTHOR(S): Tsui, F.; Mahuran, D. J.; Lowden, J. A.; Mosmann, T.; **Gravel, R. A.**
CORPORATE SOURCE: Res. Inst., Hosp. Sick Child., Toronto, ON, M5G 1X8, Can.
SOURCE: J. Clin. Invest. (1983), 71(4), 965-73
CODEN: JCINAO; ISSN: 0021-9738
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The occurrence of 3 newly identified, hexosaminidase (hex)-related polypeptides resolved by SDS-polyacrylamide gel electrophoresis of immunoppts. from [35S]**methionine**-labeled human fibroblast exts. was reported. These polypeptides, called band 2 (75,000), band 3 (70,000), and band 4 (63,000), were immunopptd. by an antiserum specific to placental hex I2. They are distinct from pre-.alpha.- (60,000) and pre-.beta.- (58,000) precursor polypeptides and the .alpha.- (56,000), .beta.a- (27,000), and .beta.b- (27,000) polypeptides of the mature hex A (.alpha..beta.a.beta.b) and hex B (2[.beta.a.beta.b]). When fibroblast exts. were chromatographed on DEAE-Sepharose, bands 2, 3, and 4 were eluted together in fractions before hex A, in a position characteristic of serum and placental hex I2 and serum hex P. Thus, bands 2, 3, and 4 may represent the polypeptides of a fibroblast hex I. The anal. of partial proteolytic digests of the radioactively labeled polypeptides revealed that bands 2 and 3, pre-.beta., and .beta.a had several peptides in common, suggesting that they are structurally related to each other. However, bands 2, 3, and 4 were present in exts. of Tay-Sachs (pre-.alpha. and .alpha. deficiency) and Sandhoff cells (pre-.beta., .beta.a, and .beta.b deficiency) and appeared later than pre-.beta. in pulse-chase expts. Hence bands 2 and 3 occur independently of pre-.beta. and .beta.a and are probably specified by different mRNAs, whether from the same gene or distinct but homologous genes.

L4 ANSWER 1 OF 2 MEDLINE
ACCESSION NUMBER: 1998169496 MEDLINE
DOCUMENT NUMBER: 98169496 PubMed ID: 9501215
TITLE: Cloning and mapping of a cDNA for **methionine synthase reductase**, a flavoprotein defective in patients with homocystinuria.
AUTHOR: Leclerc D; Wilson A; Dumas R; Gafuik C; Song D; Watkins D; Heng H H; Rommens J M; Scherer S W; Rosenblatt D S; Gravel R A
CORPORATE SOURCE: Medical Research Council Group in Medical Genetics, the Montreal Children's Hospital, McGill University Health Centre, Montreal, PQ, Canada H3Z 2Z3.
CONTRACT NUMBER: HL58955-01 (NHLBI)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Mar 17) 95 (6) 3059-64. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF025794
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980422
Last Updated on STN: 19980422
Entered Medline: 19980410

AB Methionine synthase catalyzes the remethylation of homocysteine to methionine via a reaction in which methylcobalamin serves as an intermediate methyl carrier. Over time, the cob(I)alamin cofactor of methionine synthase becomes oxidized to cob(II)alamin rendering the enzyme inactive. Regeneration of functional enzyme requires reductive methylation via a reaction in which S-adenosylmethionine is utilized as a methyl donor. Patients of the cblE complementation group of disorders of folate/cobalamin metabolism who are defective in reductive activation of methionine synthase exhibit megaloblastic anemia, developmental delay, hyperhomocysteinemia, and hypomethioninemia. Using consensus sequences to predicted binding sites for FMN, FAD, and NADPH, we have cloned a cDNA corresponding to the "**methionine synthase reductase**" reducing system required for maintenance of the methionine synthase in a functional state. The gene MTRR has been localized to chromosome 5p15.2-15.3. A predominant mRNA of 3.6 kb is detected by Northern blot analysis. The deduced protein is a novel member of the FNR family of electron transferases, containing 698 amino acids with a predicted molecular mass of 77,700. It shares 38% identity with human cytochrome P450 reductase and 43% with the C. elegans putative **methionine synthase reductase**. The authenticity of the cDNA sequence was confirmed by identification of mutations in cblE patients, including a 4-bp frameshift in two affected siblings and a 3-bp deletion in a third patient. The cloning of the cDNA will permit the diagnostic characterization of cblE patients and investigation of the potential role of polymorphisms of this enzyme as a risk factor in hyperhomocysteinemia-linked vascular disease.

L4 ANSWER 2 OF 2 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 97008193 EMBASE
DOCUMENT NUMBER: 1997008193
TITLE: Methionine and serine formation in control and mutant human cultured fibroblasts: Evidence for methyl trapping and characterization of remethylation defects.
AUTHOR: Fowler B.; Whitehouse C.; Wenzel F.; Wraith J.E.
CORPORATE SOURCE: Dr. B. Fowler, Basel University Children's Hospital, Postfach 4005 Basel, Switzerland
SOURCE: Pediatric Research, (1997) 41/1 (145-151). ISSN: 0031-3998 CODEN: PEREBL
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 007 Pediatrics and Pediatric Surgery
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB The conversion of labeled formate to methionine and serine, as a measure of remethylation of homocysteine to methionine and folate coenzyme cycling, has been studied in control and mutant human fibroblasts. Fibroblasts in monolayer culture were incubated with [14C]formate, and labeled methionine sulfone and serine were determined in hydrolysates of oxidized cell proteins. In control cells, methionine and serine were clearly measurable (n = 21, 1.7- 5.5 and 2.4-9.7 nmol/mg protein/16 h, respectively). In contrast, methionine formation was reduced in cells from patients with methylenetetrahydrofolate **reductase** (MR) deficiency (MR mutant, n = 11, 0.05-0.44), combined methylmalonic aciduria/homocystinuria [cobalamin(cbl)C/D mutant, n = 12, 0.014-0.13), and **methinine synthase** deficiency (MS mutant, n = 3,

0.04- 0.23). Furthermore, serine formation was low in cblC/D mutant (0.08-0.98) and MS mutant (0.17-0.94) cells, but normal or high in MR mutant cells (5.2- 11.4). Growth of cblC/D mutant cells in medium supplemented with high concentrations of hydroxo-cbl resulted in significant increases of both methionine and serine formation. Taken together these findings provide clear evidence for the existence of the formate to serine pathway described by W. B. Strong and V. Schirch in cultured fibroblasts and indicate that disturbed MS function due to a specific genetic disorder is associated with reduced serine formation in vitro, which reflects availability of reduced folate coenzymes. The correction of this defect by vitamin B12 alone, in cblC/D mutant cell lines, correlates well with the clinical response in the patients and fits in well with the idea that reduced availability of folate coenzymes occurs in functional MS deficiency, in agreement with the methyl trap hypothesis.